CELL CULTURE TECHNICAL PROCEDURES

XX. 100 Cell Lines and Preparation for Test

XX. 101 Lines: Use P388 **or** L1210 cells.

XX. 102 Stock Cultures: Cultivate in **a** stationary flask (cells will not adhere) in Fischer's Medium plus 10% horse serum. Twenty-four hours before test prepare **a** spinner flask culture to obtain **cells** in logarithmic phase of growth.

XX. 103 Preparation of Cell Suspension: Dilute spinner cell suspension to 6.6×10^4 cells per ml (this will be 5×10^4 cells per ml in final test mixture). Deliver 1 ml of test material and 3 ml of cell suspension to culture tubes. Incubate at 37°C for 48 hours. The baseline count (Co) will be 5×10^4.

XX. 104 Preparation of Materials: See 13.104.

XX. 200 Dose Levels: All synthetics and plant products are to be tested by weight (W), not dilution. First tests **of** synthetics and **all** priority plant fractions are **to** be scheduled at 100, 10, 1, 0.1, and 0.01 ug/ml . All plant crudes and B002 samples are tested at 100, 10, and 1 ug/ml . Fermentation products with **a** sample code of D through K may be tested by weight **or** dilution. All other fermentation products must be consistent (W or D) within the NSC core number. When tested by dilution, starting doses are 1:10, 1:100, and 1:1000 (one point pre-screens are done at **a** dilution of 1:50).

XX.300 Experimental Design: Use a common set of control tubes to evaluate materials tested at one time (about 40). Use the number of control tubes according **to** the formula $2\sqrt{n}$ where n = number of materials being tested.

XX.400 Calculations: Cell counts are made for all test and control tubes by an appropriate means: hemocytometer, Coulter counter, etc. For an automatic counter, it may be necessary to derive a calibration factor by comparison **of a** hemocytometer count of control cells with the machine count. Duplicate counts of each tube may be desirable. Routinely, a 1:20 dilution of control and test tubes is made in a medium appropriate to the cell counter being used.

XX.401 The mean value of all control tubes will be used for calculations of C.

XX.402 The mean of the experimental tubes (T) for each dose (dilution) minus the mean of the baseline (Co) divided by the mean of the control tubes (C) minus Co **give the** growth ratio (Y) at each dose level. Multiply by 100 and express as percent.

$$100 \times \frac{T - Co}{C - Co} = Y\ \%$$

XX.403 The slope is the difference in response for a one-log difference in **dose,** calculated by linear least squares regression, as follows:

A. If the growth ratios (i.e., Y values) computed for each dose are all greater than 55%, do not compute the slope. Indicate that the ED50 is greater than the maximum dose for weight formulation, or less dilute than the smallest dose for testing by dilution.

B. If the Y values are <u>all</u> less than 45%, bypass the slope calculation and indicate that the ED50 is less than the minimum dose (weights), or more dilute than the greatest dose (for dilution testing).

C. Otherwise, compute the slope and intercept of the regression line as follows. Note -- do not use more than one point from the region $Y \leq 15\%$; similarly, only one point from the region $Y \geq 85\%$ should be used.

N = number of points selected. {$\leq$number of dose levels & ≥ 2}
X_i = $\log_{10}$ of $dose_i$
Y_i = growth ratio calculated for $dose_i$
Y = $A + B \cdot X$ regression line

$$B = \text{slope} = \frac{N \bullet \Sigma(Xi \bullet Yi) - (\Sigma Xi) \bullet (\Sigma Yi)}{N \bullet \Sigma(Xi)^2 - (\Sigma Xi)^2}$$

$$A = \text{intersept} = \frac{\Sigma Yi}{N} - B \bullet \frac{\Sigma Xi}{N}$$

XX.404 The ED50 **is** the effective dose **which inhibits growth to 50% of control** growth, calculated as follows:

A . Using the slope and intercept from the regression line, compute the log of the effective dose which inhibits growth to 50% of control **growth:**

$Y = A + B \bullet X$ regression line
$50\% = A + B \bullet (\log \underline{ED50})$

log ED50 = (50-A) / B

B. For materials tested by weight, the ED50 dose is the antilog of the value computed above, i.e.:

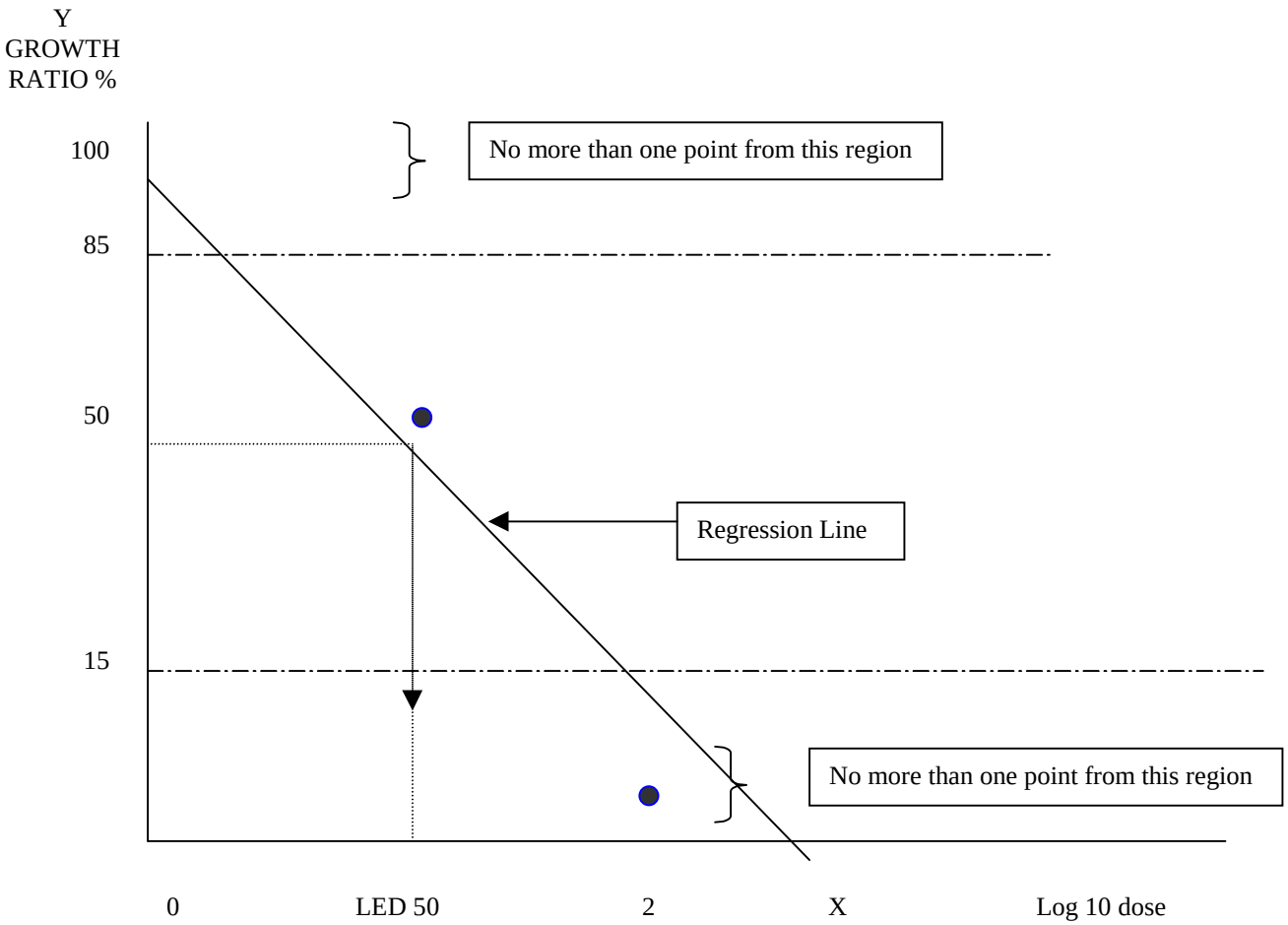
ED50 = 10 (log ED50) ug/ml

C. For materials tested by dilution, the ED50 dilution is the antilog **of** the negative log ED50, i.e.:

ED50 = 10 (-LOG ED50) dilution

EXAMPLE;

If log ED50 = -3.0,
then ED *50* = 1000 dilution
which represents a concentration of 1:1000



XX.500 Quality Control

A. The 48-hour control tubes shall show growth of at least ten times that of the baseline values.

B. The maximum allowable difference between cell counts of duplicate tubes at each dose level has not been set.

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C. The limit for reversal of Y values between consecutive dose levels has not been set.

D. The positive control compound, NSC 95441 (MeCCNU), is tested in every experiment. Quality control limits for MeCCNU are tentatively set at ED50=1.7-7.7 ug/ml.

XX.600 Test Evaluation: These parameters **have** not been set.

XX.700 Number and Interval of Dose Levels: See 13.700

XX.800 Priorities: These are as set by the DR&DP.